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Analysis of lichen thin sections by PIXE and STIM using a proton microprobe

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Abstract

In order to better understand the distribution pattern of mineral elements in lichen tissues, thin sections (15 μm) of the foliose, vagrant soil lichen *Xanthoparmelia chlorochroa* were examined using proton microprobe Particle induced X-ray emission (PIXE). This technique was used to make two-dimensional scans, with 5 μm resolution, across tissue cross sections of the test species. Element maps for Si, P, S, Cl, K, Ca, Ti, Mn, Fe, Cu, Zn, and As have been prepared. Several elements are strongly localized in the element maps. PIXE data are complimented with STIM, light micrographs, and SEM images. Preliminary data suggest that nuclear microprobe techniques may be useful in elucidating element absorption and transport mechanisms in lichens.

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1. Introduction

Particle induced X-ray emission (PIXE) spectroscopy is a sensitive, multi-element analytical technique used for determination of both minor and trace elements in a variety of samples. Biological samples are especially well suited to PIXE, including the application of PIXE in the analysis of lichens [1–3]. In these cases, element concentrations of bulk samples were measured. With the advent of scanning microprobe technology, direct micro analysis of individual biological samples is possible. Proton microprobe PIXE has been used in analyzing element distributions in a variety of biological samples, including human brain tissue, mosses, leaves, and others [4]. This technique yields not only element concentration data, but also spatial information basic to understanding physiological and biochemical relationships.

During the past several years, a number of lichens have been analyzed using PIXE for bulk element properties as part of a continuing effort to characterize air pollution effects in the Rocky Mountain region of the western United States [5,6]. Lichens are known to absorb airborne metal

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and sulfur pollutants [7,8]. Lichens are symbiotic organisms consisting of a fungus and an alga or cyanobacterium. Lichens lack vascular tissue; and frequently occur on rock, masonry, tree bark or wood, soil, or growing over other lichens. Substrate attachment patterns for lichens vary depending on species, ranging from firmly attached to vagrant (unattached). Lichens receive most of their moisture from the air with some contribution from surface moisture in some species. It is generally accepted that lichens receive most of their mineral nutrients in the form of atmospheric outwash, with minimal input from the substrate. However, the specific mechanism for delivering essential mineral nutrients to the algal layer is not clearly understood.

The study material used during this project was the foliose, vagrant, soil lichen *Xanthoparmelia chlorochroa*. Foliose lichens are dorsiventrally flattened against the substrate and in some cases they are attached to the substrate by small root-like structures call rhizines. Tissues of most foliose lichens are stratified (Figs. 2 and 3). The upper and lower surfaces of foliose lichens consist of a dense, conglutinated layer of fungal filaments. The relatively thick spongy layer between the upper and lower surfaces is called the medulla and is also made up mostly of fungal filaments. The algal layer is located in the upper portion of the medulla, immediately beneath the upper surface. The structure and availability of this species make it an ideal candidate for proton microprobe analysis.

2. Experimental

Samples of *Xanthoparmelia chlorochroa* were collected at three sites in the western United States: Mercur, Utah; Mountain View, Wyoming; and Montrose, Colorado. Lichen thalli were sectioned using a cryo microtome at −65°C with a glass knife. Sectioning was accomplished by immobilizing the sample in a drop of water and freezing it with liquid nitrogen. An attempt was made to section without immobilizing the sample in water, but sections obtained in this manner were not uniform and demonstrated a greater tendency to fragment. Sections were generally 1–2 mm long, 0.5 mm wide, and 15 μm thick. Immediately following sectioning, samples were transferred to a thin nylon film mounted on a polyethylene target frame and placed in a lyophilizer for freeze drying.

All samples were examined at the nuclear microprobe facility in the Center for Accelerator Mass Spectrometry at Lawrence Livermore National Laboratory. PIXE and STIM data were obtained using 3 MeV incident protons. A beam spot 4 μm in diameter was used, with a beam current of up to 1–2 nA. The total charge deposited to each sample was 2.4 μC. X-rays were detected with an energy-dispersive, Iglet-X, X-ray detector (EG&G Ortec, Oakridge, TN) that subtended a solid angle of ∼100 msr to the specimen and had an energy resolution of ∼160 eV at 5.9 keV. The detector was located at an angle of 135° with respect to the incident beam. Charge was collected in a biased Faraday cup located behind the sample. X-rays were recorded in list mode along with coincident beam spatial coordinates arising from scanning the beam electrostatically over the sample in a point by point raster mode.

For STIM analysis, residual ion energies were measured with a retractable, charged-particle silicon surface barrier detector located approximately 5 cm directly behind the sample and in front of the Faraday cup. Residual ion energies were recorded in list mode along with coincident beam spatial coordinates arising from scanning the beam electrostatically over the sample in a point by point raster mode. The residual energy of nineteen ions was measured at each beam location. The median value of these 19 residual ion energies was selected to represent the average residual energy of the ion beam after traversing the sample. The surface barrier detector was retracted from the path of the beam during PIXE measurements.

Element areal densities were quantified by calibrating the analysis system with thin target standards and using STIM analysis of one sample to estimate target thickness corrections. STIM analysis revealed variation in proton energy loss across a lichen cross section (see Section 3). This and the element map data suggest variation in sample density and composition. For these reasons element areal densities (mg/cm^2) with 10–20% uncertainties are given in the data table rather than

element concentrations (ppm by weight) and light element data are considered to be relative and not quantitative. X-ray spectra and STIM data were analyzed using the program PIXFIT which generated X-ray yields and sample thickness corrections.

3. Results and discussion

Of the six PIXE scans performed, only one will be discussed in this paper. This scan was of a lichen collected from a relatively pristine area in Montrose, Colorado. The scan area was 160×10 pixels, with pixel dimensions 4.88 μm by 4.92 μm. The element maps obtained by PIXE, along with the light microscopy and electron micrograph images are shown in Figs. 1–3. Element areal densities ($\mu g/cm^2$) for selected regions are given in Table 1. The upper and lower cortex, along with the dark green algal layer and the white medullary layer are clearly distinguishable in the light microscope image. The brown colored mark is specimen discoloration from proton irradiation and corresponds to the PIXE scan region. Also visible are several of the rhizinae, one of which was partially included in the scan. The scan mark was helpful in selecting the region for the SEM scan. Basic anatomical features of the thallus are indicated on the electron micrograph (Fig. 3). It is significant to note that most of the scan area is comprised of fungal hyphae, and that the algal layer is very narrow in comparison.

Several patterns in element distributions are apparent from the element maps. Elements generally seem to be most concentrated around the algal layer and in the lower cortex, especially in the rhizine. The medullary layer has lower concentrations for many elements. The nonmetal elements chlorine, phosphorus, and sulfur are most concentrated in the general area of the algal layer. These concentrations are the most pronounced for

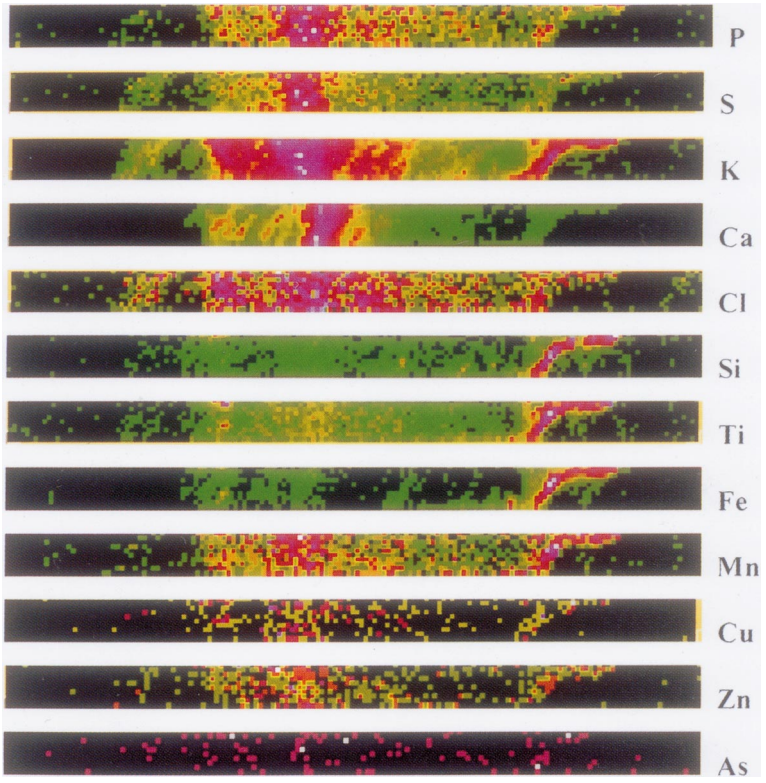

Fig. 1. Element maps of the scan indicated in Fig. 2. Concentrations decrease in the order white>purple>red>green>black.

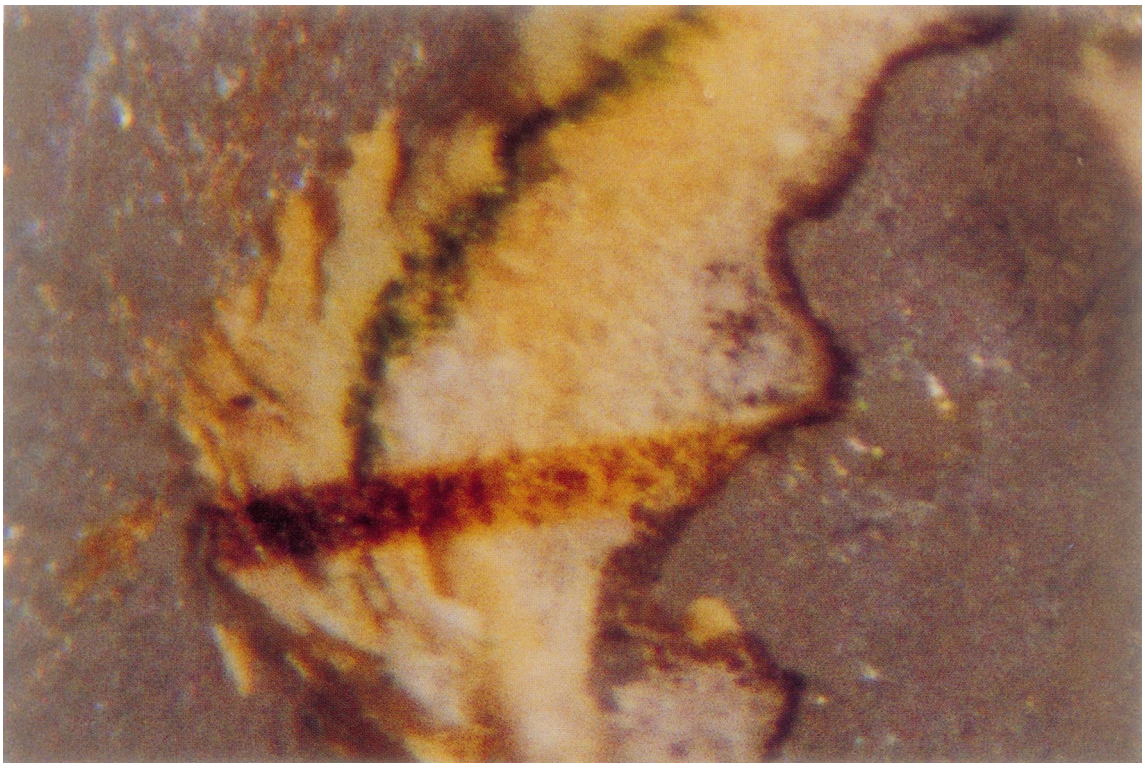

Fig. 2. Light microscope image (4X magnification) of the lichen *Xanthoparmelia chlorochroa* cross section. Note the green algal layer. The brown scorch marks are caused by proton irradiation from the scan.

phosphorus and sulfur. Chlorine is unique because it seems to be more uniformly distributed over the whole lichen. Potassium concentrations are also high near the algal layer, but the pattern differs in two respects from sulfur and phosphorus. The distribution is much broader and potassium is also highly concentrated in the rhizine. Of all the elements, calcium has the highest concentration and the most narrow concentration pattern. Calcium concentrations in bulk samples are often one weight percent or higher [5]. This and other evidence suggests that calcium levels in highly concentrated areas may reach about ten percent, which infers the presence of calcium mineralization. It is also interesting to note that the calcium concentration pattern is offset towards the medullary layer from the main concentrations of phosphorus, sulfur, and potassium. The data in Table 1 exhibit with numeric values the trends discussed above.

The element distributions discussed may be related to the bioactivity of the algal region where photosynthesis takes place and where there is ac-

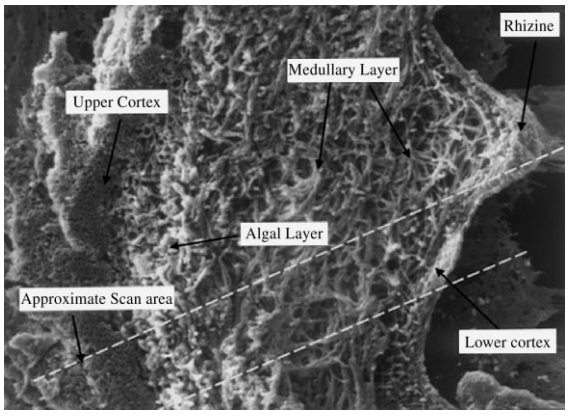

Fig. 3. SEM image (250X magnification) of the sample shown in Fig. 2. Structural layers are labeled.

Table 1
Element areal densities in selected sample regions (μg/cm^2)

Region	P	S	K	Ca	Cl	Fe	Ti	Mn	Cu	Zn
Upper cortex	1.3	1.1	18.0	45	1.1	0.52	0.067	0.012	0.013	0.031
Algal layer	5.2	4.9	36	125	1.4	0.15	0.087	0.037	0.010	0.048
Medulla	1.2	0.25	6.2	3.2	0.19	0.19	0.026	0.0048	MDL[a]	0.010
Rhizine	0.91	0.54	17	2.2	0.36	7.4	1.1	0.030	0.021	0.055
Full scan	1.7	1.5	13.9	28	0.91	0.76	0.14	0.025	0.0056	0.022
Error	Rel[b]	Rel	20%	20%	Rel	10%	10%	10%	20%	10%

[a] Below minimum detection limits.
[b] Relative, non-quantitative values used to show patterns.

tive exchange of nutrients and metabolic products between alga and fungal components The elements observed in this region are key components of biological materials and of cell transport mechanisms.

Concentrations of the transition metals manganese, zinc, and copper have patterns similar to that of potassium. These elements seem to concentrate in the rhizine and the general region of the algal layer. Another trend of interest is the behavior of elements associated with soil and rock: iron, titanium, and silicon. All these elements show large concentrations in the rhizine and on the lower cortex, with overall lower, uniform concentrations across the rest of the sample. These data suggest that these elements are not as transportable within the lichen thallus, and thus may be restricted to the contact zone between the thallus and the substrate.

A STIM scan of the Mountain View, WY, sample showed an average energy loss on the order of 170 keV. Ion beam energy losses were converted to specimen projected densities assuming the chemical composition of the biological material was $C_5H_9O_2N$ [9] and a freeze dried material density of 1.3 g/cm^3 [10]. These assumptions infer a sample thickness of 15 μm. However, in the high calcium area, the energy loss was as much as a factor of four above the average.

4. Conclusions

In this preliminary study, proton microprobe PIXE has been successfully used to analyze element distribution patterns in foliose lichens. Data show a significant degree of element partitioning in thalli of the foliose vagrant lichen *Xanthoparmelia chlorochroa*. While specific transport mechanisms are not fully understood, these data suggest that selective, efficient transport necessary for alga survival and operation may be occurring. Whether mineral nutrients are coming from the atmosphere and/or substrate remains to be determined.

5. Future work

Currently we are researching possible biochemical and physiological explanations for this element partitioning phenomenon. These efforts will include higher resolution scans with corresponding STIM scans to refine the data and support possible explanations for partitioning. In particular, higher resolution scans of the calcium region would be valuable in further characterizing its behavior. This research may also enhance efforts to use lichens in biomonitoring of air quality.

Acknowledgements

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